Comparative Study on Different Modified Techniques Used For DNA Isolation From Teeth Samples for Obtaining Optimized Output

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Abstract

DNA isolation is a process used for isolation of DNA from different types of samples using a combination of physical and chemical methods. A comparative study is made to isolate DNA from 30 teeth samples with some pre PCR and post PCR modifications in the organic Phenol: Chloroform: isoamyl alcohol, Automate Express^{TM} prepfiler BTA kit and QIAamp® DNA Investigator Kit. Our result showed significant results which will be helpful in short listing the array of these techniques.

Key Words: *DNA, RT-PCR, PCR, STR Analysis, Forensic Science*

Introduction

DNA fingerprinting analysis is used to solve the cases of murder, rape, paternity, child swapping, immigration, genealogical and medical research . DNA fingerprinting has proved to be very useful in the identification of victims of war, air crash, flood, earthquakes, Tsunami and mass disasters cases[1]. Friedrich Miescher was the first person to formulate the process of DNA isolation in 1869[2]. Various automated DNA isolation instruments have been designed by various companies to carry out the DNA isolation process in a very short time without the risk of contamination of the DNA isolates[2]. The phenol–chloroform method is conventional and the most effective method used in the various laboratories to isolate the DNA from the samples [3]. Automate Express^{TM} with BTA prepfiler kit (applied biosystem) is an automated DNA isolation process using magnetic beads during isolation[4].

The QIAamp DNA Investigator Kit can be manually operated for the isolation of DNA from a variety of biological samples. Isolation steps involve: (a) disruption of cellular membranes (b) binding of DNA to the silica-based membrane (c) washing of contaminants and (d) DNA elution. The QIAamp DNA Investigator Kit is simple and easy to use yielding of good quality of DNA[5].

UV light was the first sterilization technique used to eliminate amplification products carryover contamination[6,7]. The principle behind Ultra-violet light irradiation is the property of UV light to induce thymidine dimers and other covalent modifications of DNA that render the contaminating nucleic acid inactive as a template for further amplification[8]. The technique is simple, inexpensive, and does not require modification of existing protocols[9].

Materials and Method

Thirty three (30) teeth samples from different cases

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were used for identification related to legal assessment.

Cleaning and grinding of teeth samples

Each teeth surface was cleaned. Soft tissue, dirt from teeth surfaces were removed. Teeth fragments were immersed in distilled water for 30 sec, followed by 100% ethanol for 30 sec. and dried at 50°C for 2 hours. Teeth fragments were pulverized under sterile conditions. The resulting teeth powder was then divided into three tubes. 50 mg were placed in each tube for the standard organic isolation method, for Automate Express™ and QIAamp® DNA Investigator Kit method. Weighing of powdered teeth samples was performed in a pre-PCR weighing room under sterile conditions using a Citizen balance.

PROCEDURE

1. Phenol-Chloroform-Isoimyl Alcohol : PCI is a manual method for DNA extraction. For each sample, a total of 50mg of teeth powder was taken into 1.5 mL tube. The samples were decalcified three times with EDTA 0.5 M pH 8.0 (HIMEDIA) for 24 hours. The samples were centrifuged at 10000 rpm for 5 min. The supernatant was discarded, and the remaining teeth powders were resuspended in 1000 µl of milique water, mixed for 10 sec, and spun down at 1000rpm for 5 min. The supernatant was discarded, and the milique water wash was repeated two more times.Then, 500 µl of Forensic Buffer (HIMEDIA), 100 µl SDS (HIMEDIA) and 15 µl proteinase K (20 mg/ml) (HIMEDIA) were added and incubated at 37°C for overnight. After centrifugation, the supernatant was transferred to a 1.5 mL tube containing 500 µl of phenol. The samples were mixed and the aqueous phase was separated by centrifugation at 10000 rpm for 10 min. The supernatant was transferred to a 1.5 mL tube containing 250 µl of phenol+250 Chloroform–isoamyl alcohol (24:1) (HIMEDIA).

The samples were mixed and the aqueous phase was separated by centrifugation at 10000 rpm for 10 min and transferred to a new tube. This extraction step was followed by another extraction, this time with 500 µl of chloroform–isoamyl alcohol (24:1). The aqueous phase was mixed with 500 µl isopropanol and 50 µl 3M sodium acetate (HIMEDIA) and samples were kept to rest for 30 minutes at -20°c. Samples containing DNA were put for centrifugation at 10000 rpm for 10 min. then washed with 70% ethanol (HIMEDIA) for centrifugation at 10000 rpm for 10 minutes for two times. Final washing was done with 100% ethanol (HIMEDIA) and put to dry at room temp. for an hour then eluted with 1X TE (HIMEDIA) in thermo mixture at 65°c for 45 minutes.

2.Automate Express™ (PrepFiler BTA Kit): The AutoMate Express™ (Applied BioSystem) Nucleic Acid Extraction System is an easy-to-use benchtop instrument that enables hands-free automation of the nucleic acid extraction process. For each sample, a total of 50 mg of the teeth powder was suspended into 1.5 mL tube with 300 µl PrepFiler BTA Buffer (Applied BioSystem) provided with kit and incubated for overnight at 37°C. Next day the incubated sample was centrifuged through Lysate tubes provided with kit and put the clear lysate sample in AutoMate Express™ in instrument as per the manual provided with the instrument. After 20 min. DNA isolate was obtained[10].

3.QIAamp® DNA Investigator Kit : For each sample placed 50 mg of powdered teeth into a 1.5 ml microcentrifuge tube. To each sample added 360 µl Buffer ATL and 20 µl proteinase K(20mg/ml) (QIAGEN) and incubated overnight at 37°C. Added 300 µl Buffer AL (QIAGEN) and mixed by pulse-vortexing for 10 sec. and incubated at 70°C with shaking at 900 rpm for 10 min. Centrifuged the tubes at speed 14,000 rpm for 1 min. and transferred the supernatant to a new 1.5 ml microcentrifuge tube. Added 150 µl ethanol (100%) (HIMEDIA) and mixed by for 15 sec. Transfered the entire lysate to the QIAamp MinElute column and centrifuged at 8000 rpm for 1 min. and discarded the collection tube containing the flow-through. Added 600 µl Buffer AW1 (QIAGEN) to the QIAamp MinElute column and centrifuge at 8000 rpm for 1 min. and discarded the collection tube containing the flow-through. Added 700 µl Buffer AW2 to the QIAamp MinElute column and centrifuged at 8000 rpm for 1 min. and discarded the collection tube containing the flow-through. Added 700 µl of ethanol (100%) to the QIAamp MinElute column and centrifuged at 8000 rpm for 1 min. and discarded the collection tube containing the flow-through. Centrifuged QIAamp MinElute column at speed 14,000 rpm for 3 min. to dry the membrane completely discarded the collection tube containing the flow-through. Dried the QIAamp MinElute column at 56°C for 3 min. Applied 50 µl Buffer ATE (QIAGEN) to the center of the membrane. Incubated at room temperature (25°C) for 1 min. and centrifuged at speed 14,000 rpm for 1 min. Now the microcentrifuge tube contains 20–30 µl Buffer ATE (QIAGEN) with DNA[11].

DNA Quantification

DNA samples were quantified by realtime PCR (Applied BioSystem). The internal PCR control (IPC) included in each sample was used to detect PCR inhibitors in the DNA isolates. PCR inhibitors retard the onset of exponential amplification in the PCR, which can be detected as an increase in the cycle threshold (Ct) value for IPC. Negative controls and reagent blanks were included in every step of the study[12].

Amplification and Analysis

DNA samples obtained from all the three methods were used for STR analysis using the AmpFlSTR Identifiler Plus PCR amplification kit (Applied BioSystem). The DNA was diluted/concentrated to obtain 1 ng of DNA per µl of the DNA sample isolated with all these methods. PCR amplification consisted of a first cycle at 95°C for 11 min, 28 cycles at 94°C for 20 seconds, 59°C for 3 min and 1 cycle at 60°C for 10 min. STR fragments were analyzed on an Applied Biosystems 3500,3500 XL Genetic Analyzer (Applied BioSystem) using POP4 polymer (Applied BioSystem), Gene Scan™ 500 LIZ Size Standard (Applied BioSystem), HiDi™ Formamide (Applied BioSystem). Positive controls (provided by the manufacturer), negative controls, and reagent blank controls were analyzed in each run. The final data were analyzed by using GeneMapper ID-X v1.4 software (Applied BioSystem) to assign allele calls based on allelic ladders provided by the manufacturer with allele peak heights of at least 100 RFU.

Pre PCR modifications

Out of the 30 teeth samples subjected for DNA isolation through Organic PCI method, 18 samples gave complete DNA profiles. The 30 teeth samples were re-subjected to DNA isolation through organic PCI method. This time the incubation period was just for 4 hours at 56**°C**. After pre PCR modifications 3 more DNA isolates give a complete DNA profiles. Thus pre PCR modification gave 10% more increased DNA isolates.

Out of the 30 teeth samples subjected for DNA isolation through AutoMate Express™ (Applied BioSystem) method, 18 samples gave complete DNA profiles. The 30 teeth samples were re-subjected to DNA isolation through AutoMate Express™ (Applied BioSystem) method. This time the incubation period was just for 4 hours at 56**°C**. After pre PCR modifications 2 more DNA isolate give a complete DNA profiles. Thus pre PCR modification gave 7% more increased DNA isolates.

Out of the 30 teeth samples subjected for DNA isolation through QIAamp® DNA Investigator Kit (QIAGEN) method, 12 samples gave complete DNA profiles. The 30 teeth samples were re-subjected to DNA isolation through QIAamp® DNA Investigator Kit (QIAGEN) method. This time the incubation period was just for 4 hours at 56**°C**. After pre PCR modifications 4 more DNA isolate give a complete DNA profile. Thus pre PCR modifications gave 13% more increased DNA isolates.

Post PCR modifications

All the amplification PCR tubes, pipettes, pipette tips etc. were exposed to UV light (combination of 300-400 nm) for 10 min. followed by the addition of target DNA.

Out of 30 amplicons amplified with the DNA isolated through PCI method that were subjected to capillary electrophoresis, 19 amplicons gave complete DNA profiles while there were some allele drops in the DNA profiles of 2 amplicons. When these 30 amplicons were transferred to new UV seterlized PCR tubes and further subjected to Ultraviolet exposure in combination of 300-400nm for about 2 minutes. These 2 amplicons gave compete DNA profiles thus increasing the DNA profile up to 7%.

Out of 30 amplicons amplified with the DNA isolated through AutoMate Express™ (Applied BioSystem) method that were subjected to capillary electrophoresis, 19 amplicons gave complete DNA profiles while there were some allele drops in the DNA profiles of 1 amplicon. When these 30 amplicons were transferred to new UV seterlized PCR tubes and further subjected to Ultraviolet exposure in combination of 300-400nm for about 2 minutes. This 1 amplicon gave compete DNA profile thus increasing the DNA profile up to 4%.

Out of 30 amplicons amplified with the DNA isolated through QIAamp® DNA Investigator Kit (QIAGEN) method that were subjected to capillary electrophoresis, 14 amplicons gave complete DNA profiles while there were some allele drops in the DNA profiles of 2 amplicon. When these 30 amplicons were transferred to new UV seterlized PCR tubes and further subjected to Ultraviolet exposure in combination of 300-400nm for about 2 minutes. These 2 amplicons gave compete DNA

profile thus increasing the DNA profile up to 6%.

Results and Discussions

The quality and efficiency of a standard organic PCI DNA isolation method, Automate Express™ and QIAamp® DNA Investigator Kit were compared to obtain human DNA and short tandem repeats (STRs) profiles from 30 teeth samples. DNA samples were quantified by realtime PCR, and STR profiles were obtained using the AmpFlSTR Identifiler plus PCR amplification kit. After pre PCR modification the Organic method recovered most DNA ranging from 0.19 to 2.95 ng/ µl (average 1.43 ng/ µl) followed by Automate Express™ BTA prepfiler kit ranging from 0.16 to 2.12 ng/µl (average 1.08 ng/ µl) and QIAamp® DNA Investigator Kit ™ method recovered least DNA ranging from 0.11 to 1.98 ng/µl (average 0.84 ng/µl).

Out of 30 teeth samples tested, complete genetic profiles (15 STR loci plus amelogenin) were obtained from 21/30 (70%) DNA samples isolated using the organic extraction protocol, only 20/30 (67%) isolated with the AutoMate Express™ (Applied BioSystem) gave complete profiles while 16/30 (53%) profiles were obtained using QIAamp® DNA Investigator Kit.

After pre PCR modification there has been an increase in the DNA STR profile by 10% from 60% to 70% by using the PCI method, an increase by 7% from 60% to 67% by using the Automate prepfiler BTA kit and an increase by 13% from 40% to 53% by using the QIAamp® DNA Investigator Kit **[Figure1]**. After post PCR modification there has been an increase in the DNA STR profile by 7% from 63% to 70% by using the PCI method, an increase by 4% from 63% to 67% by using the Automate prepfiler BTA kit and an increase by 6% from 47% to 53% by using the QIAamp® DNA Investigator Kit **[Figure2]**

Rucinski et al.[13] in their study "comparison of two methods for isolating DNA from human skeletal remains for STR analysis" described that EDTA decalcification steps used in organic extraction method significantly improved the amount of DNA recovered from bone. In our study by making simple pre-PCR and post-PCR modification, there is still the possibility of obtaining for better quality DNA for generating STR profiles from human teeth. Our aim was to optimize and standardize a DNA isolation method to improve the quality and quantity of the DNA isolates from degraded and decomposed human teeth which have very little chances to provide DNA Typing evidence in Forensic Sciences.

The amount of DNA recovered was greater from the organic extraction method than from Automate Express™ and QIAamp® DNA Investigator Kit Manual method. We cannot rule out pipetting errors. Different conditions of environmental temperature, fungal, bacterial growth, humidity and the presence of inhibitors might be factors that affect the quality and quantity of DNA obtained.

The principle behind the increase in the useful DNA STR profiles through pre-PCR modification may be inhibitors are co-purified and are still present in the DNA isolates. Re-isolation of the teeth samples with some modification increases the chance of removal of these inhibitors from the previous DNA isolates increasing the chances of better STR profiles. The principle behind the increase in the useful DNA STR profiles through post-PCR modification may be the property of UV light to induce thymidine dimers and other covalent modifications of DNA that render the contaminating nucleic acid inactive as a template for further amplification.

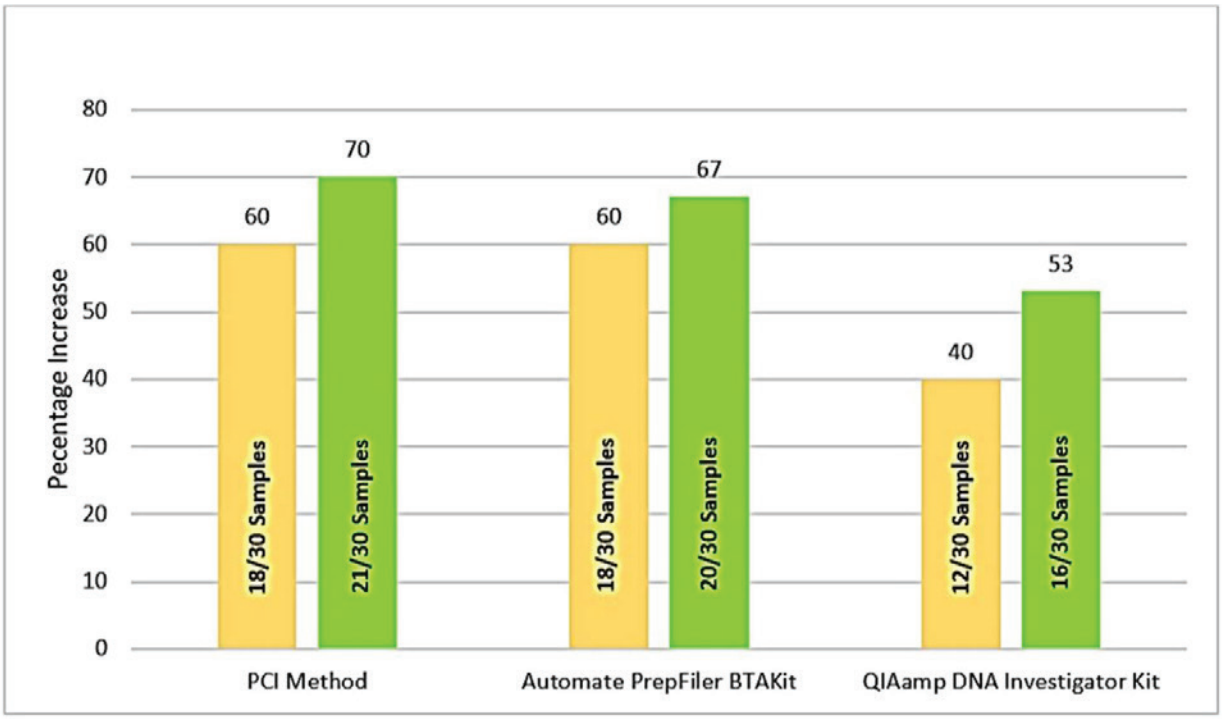

Figure1: Result obtained after pre PCR modifications in 30 teeth samples

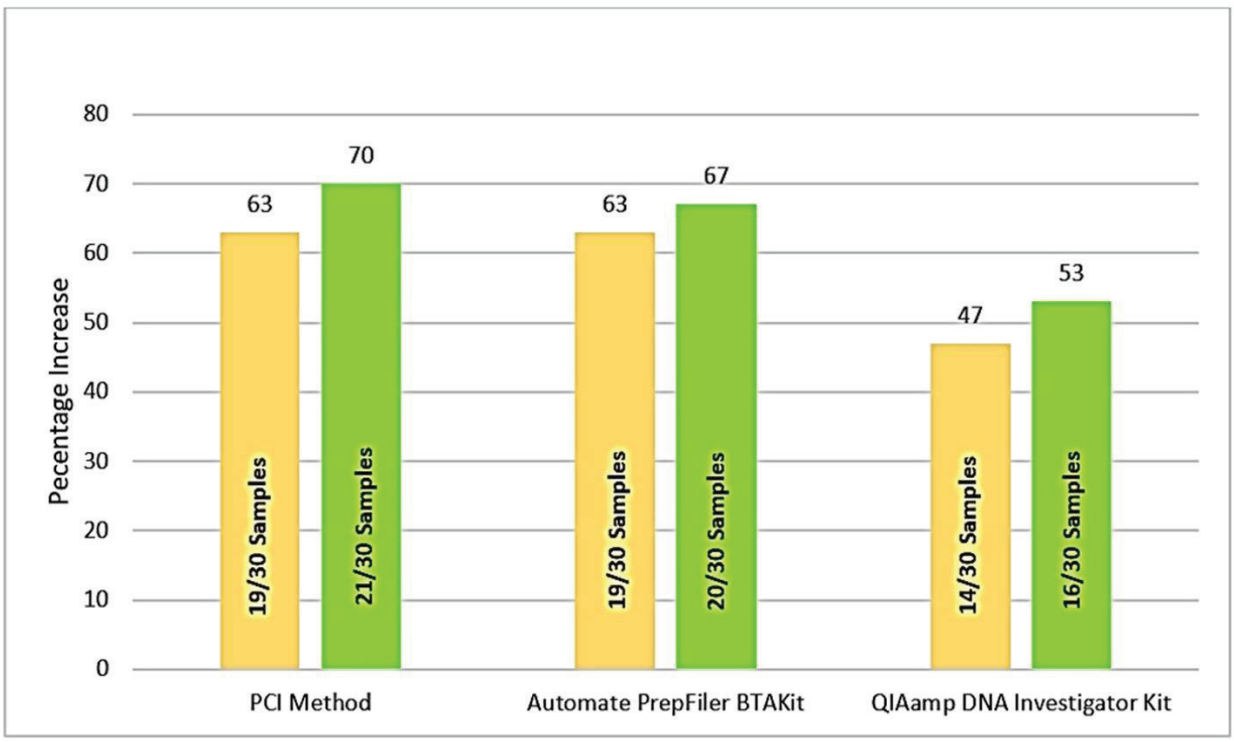

Figure2: Result obtained after post PCR modifications in 30 teeth samples

Conclusion

After thorough and extensive work on the above three DNA isolation methods, our results indicate that the organic DNA isolation method is the most suitable and reliable method of DNA isolation for obtaining DNA quantity and DNA quality followed by Automate Express™ and then QIAamp® DNA Investigator Kit™ method. The present study has proved that simply by making some pre-PCR and post-PCR modifications these three techniques still hold the potential for providing better results.

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